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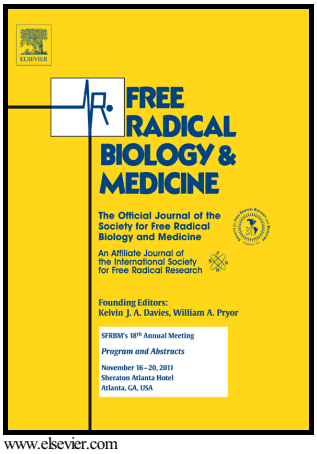

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Circadian control of p75 neurotrophin receptor leads to alternate activation of Nrf2 and c-Rel to reset energy metabolism in astrocytes via brain-derived neurotrophic factor

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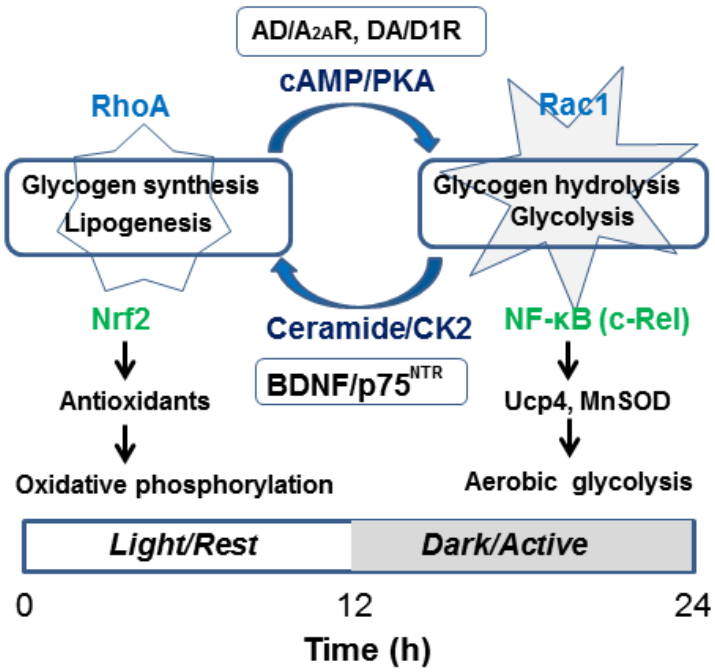
List of Abbreviations

BDNF, brain-derived neurotrophic factor; CK2, casein kinase 2; Cry, cryptochrome; CREB, cAMP-response elements binding protein; DARPP-32, dopamine- and cAMP-regulated phosphoprotein of 32 kDa; FoxO1, forkhead box protein O1; Gbe1, glycogen branching enzyme 1; GEF, guanine nucleotide exchange factor; GLUT1, glucose transporter 1; GSK3β, glycogen synthase kinase-3 β; Keap1, Kelch-like ECH-associated protein 1; mTORC1, mammalian target of rapamycin complex 1; NGF, nerve growth factor; NOX, NADPH oxidase; Nrf2, nuclear factor-E2-related factor 2; $p75^{NTR}$, p75 neurotrophin receptor; Pak1, p21-activated kinase 1; Per2, period 2; PIP3, phosphatidylinositol-3,4,5-triphosphate; PP1, protein phosphatase-1; PTEN, phosphatase and tensin homologue; RhoGDI, Rho GDP dissociation inhibitor; SCN, suprachiasmatic nucleus; SQSTM1, sequestosome 1; Trk, tropomyosin-related kinase; Ucp, uncoupling protein; ZT, zeitgeber time

Abstract

Circadian clock genes regulate energy metabolism partly through neurotrophins in the body. The low affinity neurotrophin receptor $p75^{NTR}$ is a clock component directly regulated by the transcriptional factor Clock:Bmal1 complex. Brain-derived neurotrophic factor (BDNF) is expressed in the brain and plays a key role in coordinating metabolic interactions between neurons and astrocytes. BDNF transduces signals through TrkB and $p75^{NTR}$ receptors. This review highlights a novel molecular mechanism by which BDNF via circadian control of $p75^{NTR}$ leads to daily resetting of glucose and glycogen metabolism in brain astrocytes to accommodate their functional interaction with neurons. Astrocytes store glycogen as an energy reservoir to provide active neurons with the glycolytic metabolite lactate. Astrocytes predominantly express the truncated receptor TrkB.T1 which lacks an intracellular receptor tyrosine kinase domain. TrkB.T1 retains the capacity to regulate cell morphology through regulation of Rho GTPases. In contrast, $p75^{NTR}$ mediates generation of the bioactive lipid ceramide upon stimulation with BDNF and inhibits PKA activation. As ceramide directly activates PKCζ, we discuss the importance of the TrkB.T1-$p75^{NTR}$-ceramide-PKCζ signaling axis in the stimulation of glycogen and lipid synthesis and activation of RhoA. Ceramide-PKCζ-casein kinase 2 signaling activates Nrf2 to support oxidative phosphorylation via upregulation of antioxidant enzymes. In the absence of $p75^{NTR}$, TrkB.T1 functionally interacts with adenosine $A_{2A}R$ and dopamine D1R receptors to enhance cAMP-PKA signaling and activate Rac1 and NF-κB c-Rel, favoring glycogen hydrolysis, gluconeogenesis and aerobic glycolysis. Thus, diurnal changes in $p75^{NTR}$ levels in astrocytes resets energy metabolism via BDNF to accommodate their metabolic interaction with neurons.



Graphical Abstract

Key words: circadian rhythm; clock genes; astrocytes; brain-derived neurotrophic factor; TrkB; $p75^{NTR}$; sequestosome-1; Per2; glycogen; Nrf2;c-Rel; RhoA; Rac1

Contents

1. Introduction

The master biological clock, which entrains the whole body clock, is believed to reside in the suprachiasmatic nucleus (SCN) in mammals. Although the master clock entrains the whole body rhythm, peripheral tissues also express core clock transcription factors Clock and Bmal1 which regulate expression of clock genes including Period (Per) and Cryptochrome (Cry) proteins. Complexes of Per and Cry proteins repress Bmal1- and Clock-mediated transcription forming a negative feedback loop, which regulates nearly a 24 h self-sustained rhythm including energy metabolism [1,2](Fig. 1 A).

Period2 (Per2) plays a key role in the regulation of metabolic and cardiovascular circadian rhythms [3,4]. The peripheral clock in nocturnal animals has a time shift from the master clock in the SCN to adapt to increases in energy expenditure during the

dark/active phase. Per2 clock gene expression significantly differs between the SCN and other parts of brain and peripheral tissues in rat [5,6]. Notably, the peak of Per2 protein is significantly delayed to that of mRNA. Immunostaining of brain sections show that the Per2 protein rhythm is synchronized among cells including neurons and glial cells. Per2 protein levels peak around the Zeitgeber time 1 (ZT1, early light/rest phase) and exhibit a sharp nadir around ZT13 (early dark/active phase) in the basolateral amygdala, the dentate gyrus and the dorsal striatum [5,7] (Fig. 1 B).

Neurotrophins such as brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) are produced in various neuronal and non-neuronal cells, leading to autocrine and paracrine regulation of energy metabolism to support cell survival, growth, differentiation and other functions (reviewed in [8-10]). The diverse actions of neurotrophins are mediated through two different transmembrane receptors, the common low affinity p75 neurotrophin receptor ($p75^{NTR}$) and neurotrophin-specific high affinity Trk (tropomyosin-related kinase) A, B and C receptors. The former receptor belongs to the tumor necrosis factor receptor superfamily [11]. Recently, Baeza-Raja et al. found that *p75^{ntr}* gene expression is directly controlled by the heteromeric Clock:Bmal1 master circadian transcription factor through its E-box enhancers (Fig. 1 A) and that the phases of $p75^{NTR}$ and Per2 mRNAs are synchronized with peaks at around ZT16 in mouse liver [12]. Compared to Per2 protein, there are no precise data showing the daily variation of $p75^{NTR}$ protein expression levels in brain areas. However, the transcription rates of both $p75^{NTR}$ and Per2 genes via Clock-Bmal1 will be maximal around ZT13 when the Per2 protein levels are minimal [5,7] (Fig. 1B). Considering Per2 mRNA and protein respectively peaked around ZT19 and ZT1 in dorsal striatum [7], we can predict that the $p75^{NTR}$ protein levels will peak between late dark/active phase and early light/rest phase in the brain areas. To discuss detailed daily variation of BDNF-TrkB.T1-$p75^{NTR}$ signaling pathways, precise determination of BDNF, TrkB.T1 and $p75^{NTR}$ protein levels as well as their distribution and activation states in astrocytes in the brain areas will be required.

$p75^{NTR}$-mediated signaling plays a key role in daily changes in energy metabolism in $p75^{NTR}$ expressing tissues including liver, muscle, adipose and brain. Notably, $p75^{NTR}$ positively regulates lipogenesis in the liver and loss of $p75^{NTR}$ in mice results in the

resistance to high fat diet-induced obesity with enhanced insulin sensitivity [13,14]. However, the detailed signaling pathways regulated by $p75^{NTR}$ remain to be fully elucidated.

As BDNF is enriched in adult brain, with particularly high levels in the hippocampus and cerebral cortex, it is recognized as an important regulator of neuronal plasticity and memory formation (reviewed in [15,16]). BDNF acts on both neurons and astrocytes and regulates formation of neuronal synapses as well as glucose and lipid metabolism in astrocytes, thereby underpinning metabolic interactions between astrocytes and neurons (reviewed in [9,10,17,18]) (Fig. 1 C).

In this review, we focus on the molecular mechanisms by which BDNF-mediated signaling pathways are affected by daily variation in $p75^{NTR}$ levels in astrocytes. We highlight the differential signaling pathways modulated by the TrkB-$p75^{NTR}$ complex versus TrkB without $p75^{NTR}$ in the regulation of energy metabolism. Notably, the metabolic changes are coupled with dynamic alterations in cell morphology and oxygen metabolism in astrocytes. These changes in astrocytes are critical for time-dependent sharing of oxygen and glucose with neurons.

2. Astrocytes metabolically support neurons

Glucose is the predominant energy substrate of the central and peripheral nervous system. The majority of glucose is transported to brain via the facilitative transporter GLUT1 expressed in capillary endothelial cells and astrocytes (reviewed in [19]). Neurons express GLUT3 in addition to GLUT1 [20]. Compared to GLUT1, GLUT3 has a higher affinity for glucose and a significantly greater capacity to transport glucose, providing neurons with preferential access to available glucose (reviewed in [21]). In the mammalian brain, astrocytes exceed the number of neurons and are intimately involved in signal transmission, responding to and influencing neuronal activity. Consequently, astrocytes play important roles in synaptic function and the delivery of nutrients from the circulation to neurons (reviewed in [22-26]).

Astrocytes store glycogen as an energy store in times of nutritional sufficiency for utilization in times of need. Transported glucose and glycogen derived glucose-6-phosphate are initially converted to pyruvate during glycolysis and pyruvate

is completely oxidized in the tricarboxylic acid (TCA) cycle. When neurons are activated astrocytes synthesize neurotransmitter glutamate which is transferred to neurons through glutamine-glutamate cycle to support neuronal activity. Glycolytically derived energy is required for this process in both neurons and astrocytes. As glycolysis raises cytosolic redox potential (NADH > NAD^+), excess pyruvate, not used for TCA cycle, is preferentially converted to lactate as this reaction oxidizes NADH back to NAD^+ (reviewed in [27]).

Astrocytes flexibly change energy metabolism such as glycogen synthesis and hydrolysis, and increase in glycolysis in response to nutritional status, hormonal stimulation and the energy demands of neurons and astrocytes themselves (reviewed in [28,29]. For example, intense physical exercise is known to cause depletion of glycogen in skeletal muscles and liver, and under such conditions rat brain glycogen also significant decreases with increase in production of lactate [30]. Serotonin plays a key role in the glycogen mobilization during exercise and the brain glycogen recovers in 6 h after cessation of exercise [31,32]. Brain glucose metabolism also changes during the sleep-wake cycle. During wakefulness, norepinephrine-dependent high rates of aerobic glycolysis and lactate production in both neurons and astrocytes, and glycogen hydrolysis in astrocytes can be observed. Lactate in turn potentiates norepinephrine release by noradrenergic terminals (reviewed in [33]).

Although astrocytes change their metabolism in response to extracellular stimuli, the cellular clock governs the daily rhythm of metabolism to save or efficiently expend energy. As the $p75^{NTR}$ receptor is a clock component and BDNF is the major neurotrophin, the BDNF-$p75^{NTR}$ signaling axis seems to play an important role in the daily rhythm of brain metabolism and the metabolic cooperation between astrocytes and neurons. Disturbances in neuron-astrocyte metabolic cooperation have been linked with impaired long-term memory, synaptic plasticity, cognition and ultimately neurodegeneration (reviewed in [34-36]). Thus, understanding how BDNF coordinates metabolic interactions between astrocytes and neurons has emerged as an important research field.

3. Molecular functions of BDNF receptors expressed in astrocytes

The diverse actions of BDNF are mediated through TrkB and $p75^{NTR}$ receptors. The TrkB receptor has a higher affinity for BDNF than $p75^{NTR}$, but the two receptors usually work together in multiple intracellular signaling pathways triggered by BDNF. Notably, astrocytes exclusively express a truncated TrkB.T1, which lacks the intracellular tyrosine kinase domain and hence transduces restricted intracellular signals compared to the full size TrkB [37]. This is an important point in comparing BDNF signaling pathways in astrocytes with those in neurons, which express full size TrkB in addition to TrkB.T1.

3.1 TrkB.T1 signaling regulates cell morphology via RhoGTPase

A notable function of BDNF is regulation of cell morphology through RhoGTPases, which rearrange the actin cytoskeleton through interaction with specific effectors (reviewed in [38,39]). Ohira et al. [37,40] found that TrkB.T1 associates with the Rho GDP dissociation inhibitor-1 (RhoGDI-1). RhoGDIs bind to geranyl lipids, attached at C-terminus of Rho GTPases and maintain them inactive (reviewed in [41,42]). Activation of Rho GTPases requires release from GDI to membrane and replacement of GDP to GTP by specific guanine nucleotide exchange factors (GEFs) before interaction with their effectors (Fig. 2 A and B).

Specific activation of Rho GTPases can be achieved by phosphorylation of GDI by different kinases. Phosphorylation of GDI by PKCα at Ser-34 induces specific release of RhoA due to lowering its affinity for RhoA [43]. In contrast, phosphorylation of GDI with p21-activated kinase 1 (Pak1) specifically releases Rac1 [44] (Fig. 2 B).

Cell shape changes are linked with the alterations in energy metabolism as will be discussed further in Sections 4.1 and 5.3. Previous studies show that an increase in cellular cyclic AMP (cAMP) induces a morphological change of an astrocyte from an epithelial-like to stellate morphology [45,46]. It has been reported that BDNF stimulation induces elongation of astrocytic processes [37,47], suggesting BDNF-TrkB.T1-mediated signals can induce an increase in cAMP and Rac1 activation (Fig. 2 C). The elongation of cell processes will increase contact areas with neuronal synapses. It is therefore important to clarify the mechanism by which BDNF-TrkB.T1 regulated morphological changes in astrocytes affect metabolism and their functional

interaction with neurons.

3.2 Two notable functions of p75^{NTR}

Baeza-Raja et al. [13] recently reported that p75^{NTR} directly interacts with PKA and inhibits its activation. This effect of p75^{NTR} is apparently not related to neurotrophins. Deficiency of p75^{NTR} accompanies increased cAMP and PKA activity causing an increase in lipolysis and energy expenditure in adipocytes [13]. These authors further concluded that the presence of p75^{NTR} favors lipogenesis in rat, but it remains unclear whether the neurotrophin-mediated signaling pathways contribute to lipogenesis in adipose tissues and liver. Although PKA also plays a key role in glycogen hydrolysis as will be discussed in Section 5.3, the effects of p75^{NTR}-mediated signaling on glycogen metabolism have not been reported.

The second notable function of p75^{NTR} is the generation of the lipid signaling molecule ceramide upon stimulation with neurotrophins involving activation of neutral sphingomyelinase [48]. Ceramide is involved in various biological responses including proliferation, differentiation and apoptosis [49]. As ceramide induces apoptosis at high concentrations, overstimulation of p75^{NTR} leads to apoptosis in neurons and glial cells [50]. However, the effects of physiological concentrations of ceramide generated through p75^{NTR} on metabolism and regulation of GTPases in astrocytes are not clear at present.

A ceramide sensitive atypical protein kinase PKCζ mediates an important part of ceramide function [49]. Ceramide binds to and regulates kinase activity of PKCζ in a biphasic manner [51], noting that ceramide at 0.5-60 nM activates PKCζ, whereas concentrations above 60 nM decrease kinase activity to basal levels. Based on these and other studies, we discuss possible mechanisms by which p75^{NTR} participates in the regulation of metabolism and RhoGTPases via BDNF.

4. Neurotrophin signaling via p75^{NTR}

To discuss the effects of variation in p75^{NTR} levels on metabolism, it is important to compare differences in metabolism between the two conditions, e.g. the presence and absence of p75^{NTR}. The first step is to examine the signaling pathways regulated through

the $p75^{NTR}$ receptor stimulation.

4.1. Activation of RhoA via $p75^{NTR}$ signaling

Although previous studies show $p75^{NTR}$ can activate RhoA through direct interaction with RhoGDI following stimulation with myelin-associated glycoprotein in neuronal cells [52,53], the effects of BDNF-TrkB.T1-$p75^{NTR}$ signaling pathway in the modulation of Rho GTPases in astrocytes remain to be clarified. We propose a possible signaling pathway that $p75^{NTR}$-mediated signals specifically activate RhoA via modification of RhoGDI by PKCα in astrocytes. We suggest $p75^{NTR}$-ceramide-PKCζ signaling can induce Ca^{2+}-influx via membrane depolarization leading to activation of PKCα (Fig. 3 A).

A notable function of PKCζ is to modulate the membrane potential through regulation of *Shaker* type voltage-activated potassium (K_v1) channels [54]. K_v1 channels are composed of membrane-spanning α subunits and cytoplasmic auxiliary β subunits, $(K_v\alpha)_4(K_v\beta)_4$ (Fig. 3A). A signal adaptor protein sequestosome-1 (SQSTM1/ZIP/p62/A170), scaffolds PKCζ to help phosphorylate β-subunit of K_v1 channels [54-56]. Furthermore, SQSTM1 is known as a regulator of $p75^{NTR}$-mediated signaling. It directly interacts with $p75^{NTR}$ and helps to transduce NGF-mediated signals in rat pheochromocytoma PC12 cells [57,58]. It was also shown that the NGF treatment upregulates SQSTM1 expression and activates PKCζ leading to modulation of K_v1 channels in PC12 cells [59,60].

Astrocytes express $K_v\alpha1.5$ type channels as the major *Shaker* type potassium channel [61]. Notably, the same PKCζ-SQSTM1-$K_v1.5$ signaling complex exists in mouse pulmonary arterial smooth muscle cells and functions in association with a sensor system to detect hypoxia [62]. Acute hypoxia-induced PKCζ-SQSTM1 mediated phosphorylation of $K_v1.5$ β subunits causes inhibition of $K_v1.5$ currents and local membrane depolarization leading to vasoconstriction of pulmonary arteries [62]. The hypoxia-induced inhibition of $K_v1.5$ currents are largely depended on PKCζ activation and increased generation of ceramide by neutral sphingomyerinase [63,64]. Local membrane depolarization increases Ca^{2+} influx through L-type voltage-gated calcium channels leading to vasoconstriction through RhoA activation in the smooth muscle

cells [62,65]. Depolarization-mediated Ca^{2+} influx activates PKCα and the ERK-dependent RhoGEF-H1 resulting in RhoA activation [66]. In astrocytes, the PKCζ-SQSTM1-K_v1.5 signaling complex can be associated with TrkB.T1-$p75^{NTR}$ receptor complex and transduce signals when ceramide is generated following BDNF binding to the receptor (Fig. 3 A).

4.2 Activation of casein kinase 2 and Nrf2 via $p75^{NTR}$ signaling

Kosaka et al. [67] showed NGF treatment induces activation of transcription factor Nrf2 in PC12 cells. Nrf2 is a master regulator for the cellular defense against oxidative stress (see recent FRBM Special Issue on Nrf2 [68]). Nrf2 positively regulates expression of many genes including detoxifying phase II and antioxidant enzymes and proteins [69-71]. However, the precise mechanism how NGF activates Nrf2 has not been elucidated. Meanwhile, neurotrophins such as NGF, BDNF and neurotrophin-4 (NT-4) activate casein kinase 2 (CK2) in rat hippocampal slices or neurons [72,73] and that $p75^{NTR}$ is required for CK2 activation in mouse hippocampal neurons following stimulation with NGF [74] (Table 1, part 1). CK2 is highly expressed in the mammalian brain and has many substrates important for neuronal or glial homeostasis and synaptic signaling processes (reviewed in [75]).

Activation of Nrf2 can be regulated via multiple mechanisms, and the Kelch-like ECH-associated protein 1 (Keap1)-mediated indirect regulation of Nrf2 stability by electrophiles is the most characterized pathway [76]. Direct modifications of Nrf2 by kinases also regulate stability and nuclear translocation of Nrf2. For instance, Ca^{2+}-regulated PKC directly phosphorylates Nrf2 and transitory enhances ARE-mediated gene expression [77]. Additionally, Nrf2 has multiple phosphorylation sites for CK2 in the Neh4 and Neh5 transcription activation domains and phosphorylation by CK2 stabilizes/activates Nrf2 [78,79]. CK2-mediated activation of Nrf2 was previously observed in cells stimulated with oxidized phospholipids and *tert*-butyl-hydroquinone (tBHQ) [79,80] (Table 1, part 2). Although tBHQ can also target Keap1 Cys-151 to activate Nrf2 [76], tBHQ-CK2-Nrf2 axis contributes much more for the Nrf2 stabilization [78-80]. It is noted that a kinase inhibitor LY294002 is known as a PI3K inhibitor but also potently inhibits CK2, and that Afonyuskin et al.

[80] showed PI3K-specific inhibitor wortmannin hardly inhibits Nrf2 activation by oxidized phospholipids in human endothelial cells. These studies strongly support an idea that neurotrophin-$p75^{NTR}$-CK2 axis induces Nrf2 activation.

CK2 contributes to the growth and survival of malignant gliomas and has been considered as a therapeutic target in these tumors [81-83]. Although direct correlation between CK2 activity and Nrf2 activation among gliomas is currently unproved, Nrf2 target genes are elevated in some of the gliomas [84]. Interestingly, Cong et al. (2013) [85] showed that the kinase inhibitor LY294002 suppressed Nrf2 activity in human glioblastoma U251 cells (Table 1. Part 2). The authors argued that PI3K signaling pathway partly regulate Nrf2 activation, however, it is possible that CK2 activity contributes enhanced Nrf2-mediated defense system in the glioblastoma cell line as LY294002 also inhibits CK2 activity,.

Astrocytes have a high capacity to upregulate the Nrf2/ARE system. Kraft et al. stimulated fresh brain astrocytes from mouse embryos with tBHQ, and observed about 80 genes were upregulated through an ARE-dependent manner [86]. Those included typical Nrf2 target genes important in glucose and fatty acid metabolism, glutathione synthesis and detoxification [86]. Genes encoding Glucose-6-phosphate dehydrogenase [87], glycogen branching enzyme [88] and SQSTM1 (A170) [70] were included in the activated genes by tBHQ in the astrocytes [86]. Glucose-6-phosphate dehydrogenase is a key enzyme for the production of NADPH through the pentose phosphate pathway [86]. NADPH is important not only for reductase systems but also for fatty acid and cholesterol synthesis. Recent studies have revealed that Nrf2 is indispensable for mitochondrial activity such as fatty acid oxidation and ATP production and for mitochondrial integrity by promoting mitophagy (reviewed in [89,90]).

4.3 Ceramide-mediated CK2 and Nrf2 activation

The precise mechanism of CK2 activation by $p75^{NTR}$-mediated signaling is not known. We suggest the importance of PKCs for CK2 activation as a previous study shows conventional and atypical PKCs including PKCα and PKCζ are able to phosphorylate and activate CK2α and CK2β [91]. Notably, NGF treatment actually produces ceramide, and C6-ceramide replaces NGF in the protection of sympathetic

neurons [92]. In another study, supplementing cell-permeable short-chain C2-ceramide to the culture medium provoked Nrf2/ARE signaling pathways in rat primary astrocytes [93]. Therefore, we propose that $p75^{NTR}$-ceramide activated PKCζ and depolarization/Ca^{2+}-influx activated PKCα contribute in activation of CK2/Nrf2 pathway following treatment with BDNF and other neurotrophins in astrocytes (Fig. 3B).

Although the mechanism how tBHQ activates CK2 is not known, other Nrf2 activating agents such as low density lipoproteins and/or oxidized phospholipids are reported to stimulate sphingomyelin-ceramide pathway in smooth muscle cells, endothelial cells and macrophages [94-96]. These cells are able to sense oxidized lipids through increase in ceramide generation, suggesting a possible link of the ceramide-PKCζ system with CK2/Nrf2 activation by low levels of oxidized phospholipids. It is also noted that Nrf2 activating natural phenolic compounds resveratrol and curcumin are known to produce ceramide in cells and that their anti-cancer activity partly depends on ceramide-induced growth inhibition and apoptosis [98-102] (Table 2).

Notably, the activation of Nrf2 by $p75^{NTR}$-CK2 axis is neither mediated through Keap1 modification nor directly related with oxidative stress, but is programed by the cellular clock which predicts and prepares forthcoming metabolic changes. Of course many other Nrf2 activating metabolites will be produced during a high cellular activity, but the prior upregulation of Nrf2-regulated gene products via $p75^{NTR}$-CK2 axis will reduce production of insulting metabolites.

4.4. Expected metabolic changes via $p75^{NTR}$-CK2 signaling

Currently, studies on the roles of $p75^{NTR}$ and CK2 in the energy metabolism in normal astrocytes in the brain are limited. However, we can speculate from previous studies with cultured cells and tissues that activation of CK2 could phosphorylate and inactivate PTEN (phosphatase and tensin homologue) causing an increase in PIP3 levels resulting in Akt/PKB activation [103,104]. CK2 also enhances Akt activity through a direct interaction [105], and notably Akt can activate mTORC1 leading to lipogenesis (reviewed in [106]). Akt phosphorylates and inhibits glycogen synthase kinase-3 β

(GSK3β) [107]. These signaling pathways are also expected to occur via CK2 activation in astrocytes (Fig. 3B).

As PKA plays an important role in glycogen hydrolysis, inhibiting PKA by $p75^{NTR}$ will benefit for glycogen synthesis. Protein phosphatase-1 (PP1), which is indirectly inhibited by PKA [108], plays a key role in glycogen synthesis through activation of glycogen synthase and inactivation of glycogen phosphorylase kinase and glycogen phosphorylase (reviewed in [109]). Additionally, inhibition of GSK3β via the CK2 signaling pathway will partly contributes to glycogen synthesis (Fig. 3B).

5. TrkB.T1-mediated regulation of metabolism

In the absence of $p75^{NTR}$, BDNF-TrkB.T1 signaling pathways are quite different from those governed by ceramide and PKA inhibition. As the TrkB.T1 receptor lacks a tyrosine kinase domain, BDNF-mediated signaling through this receptor is restricted. Therefore, cross-talk of TrkB.T1 with other receptors that activate PKA is thought to influence the transduction of BDNF signals.

5.1 Crosstalk of TrkB.T1 with adenosine A_{2A} receptor

In neurons, interaction of TrkB with the adenosine $A_{2A}R$ receptor has been reported [110,111]. Interestingly, adenosine stimulation via $A_{2A}R$ can induce TrkB signaling in the absence of BDNF. Moreover, BDNF can facilitate $A_{2A}R$ signaling in neurons via transactivation. It has been suggested that $A_{2A}R$-mediated activation of PKA induces translocation of TrkB receptors to lipid rafts [111]. In addition to BDNF, adenosine is a well-known modulator of synaptic maturation, plasticity and signaling [112]. Adenosine can be released from presynaptic vesicles but an increase in extracellular adenosine can also be produced from released ATP by ecto-nucleotidases (reviewed in [113,114]). The $A_{2A}R$ receptor is associated with $G_{\alpha s}$ protein and upregulates cAMP via activation of adenylyl cyclase. Astrocytes mainly express $A_{2A}R$ which contribute to neuronal excitability [115]. Based on these studies, we predict that TrkB.T1 also functionally interacts with $A_{2A}R$ in astrocytes (Fig. 3).

5.2 Functional interaction of TrkB.T1 with dopamine D1 receptor

In addition to $A_{2A}R$, dopamine D1 receptor (D1R) also functionally interacts with TrkB in neurons. Iwakura et al. [116] studied the mechanism by which dopamine via D1R regulates neurite outgrowth and morphological changes in primary neurons. They found that a D1R agonist increased TrkB-mediated signaling through enhanced surface expression of TrkB. D1R also associates $G_{\alpha s}$ like $A_{2A}R$ and activates cAMP/PKA signaling upon stimulation with dopamine. As astrocytes express D1R at high levels [117], dopamine may also affect TrkB.T1 functions.

Dopamine is a neurotransmitter involved in modulation of attention, motivation, learning, and memory consolidation [118,119]. Mid brain dopaminergic neurons send numerous projections to the prefrontal cortex and dorsal and ventral striatum, and diffusely released dopamine participates in both synaptic and extra-synaptic transmission [120,121]. Dopamine enhances glycogenolysis in brain [122]. Application of dopamine to the culture medium (1 μM) actually induces activation of the D1R-PKA signaling pathway in astrocytes within a few minutes leading to an increase in glycogen hydrolysis and glycolysis [123]. Notably, dopamine levels in the dorsal striatum exhibit a clear daily rhythm: minimal around ZT6 and maximal around ZT18 [7]. These results indicate that DA released from dopaminergic neurons could act on astrocytes to boost glycogen hydrolysis more efficiently during the dark/active phase compared to the light/rest phase.

5.3 PKA enhances glycogen hydrolysis and cell process extension

PKA plays a key role in glycogen hydrolysis. PKA phosphorylates DARPP-32 which associates and inhibits PP1 [108]. PKA also phosphorylates glycogen synthase and glycogen phosphorylase kinase, which activates glycogen phosphorylase (reviewed in [109]) (Fig. 4 A). Additionally, PKA inhibits lipogenesis through direct phosphorylation of sterol regulatory element-binding protein (SREBP)-1a and -1c [124] and indirectly through mTORC1 inhibition [125].

Rac1 can be activated by cAMP/PKA signaling [126,127]. Activated Rac1 associates its effector p21-activated kinase 1 (Pak1), which selectively releases Rac1 from the TrkB.T1-associated RhoGDI (Fig. 2 B and 4 B). Additionally, PKA can phosphorylate RhoA at Ser-188 to increase its affinity for RhoGDI resulting in RhoA inhibition [128]

(Fig. 4 B). The PKA-dependent reorganization of F-actin cytoskeleton via GTPases facilitates plasma membrane localization of the predominant water channel aquaporin 4 (AQP4), and stable cAMP upregulation induces an increase in AQP4 total protein expression causing enhancement in water influx into astrocytes [129,130]. Increase in cell volume called swelling due to alteration of AQP4 expression and Rac1 activation will help extend astrocytic processes via filopodia and lameripodia formation resulting in the increase in astrocytic coverage over neuronal synapsis.

5.4 PKA activates NF-κB c-Rel

Another function of PKA is specific activation of a NF-κB component c-Rel. The c-Rel pathway induces expression of pro-survival factors such as Bcl-xL, manganese superoxide dismutase (MnSOD) and uncoupling protein 4 (Ucp4) in neuronal cells [131,132]. PKA inhibits degradation of IκB-α and suppresses IκB-α-dependent expression of IL-2, while increased synthesis of c-Rel and enhances IL-4 expression in T cells [133]. PKA enhances nuclear localization of c-Rel presumably by phosphorylation in T cells [134].

Uncoupling proteins located in the inner membrane of mitochondria have been implicated in the suppression of oxidative phosphorylation and thermoregulation. Diminished ATP production is effectively compensated by enhanced glycolysis. A recent study by Ho et al. [132] showed that H_2O_2 activates c-Rel leading to induction of *Ucp4* gene expression. Ucp4 is a brain-specific ucp expressed in astrocytes and neurons [135]. Upregulation of Ucp4 via c-Rel reduces production of H_2O_2 from mitochondria and thereby protects cells from oxidative damages while increasing lactate production in astrocytes [136]. As described above (Fig. 4 B), PKA activates Rac1 GTPase, which is known to activate NADPH oxidases (NOXs) [137,138]. NOX2 and NOX4 are widely expressed in central nervous system including neurons and astrocytes [139]. Although H_2O_2 activates c-Rel, the contribution of the Rac1-NOX signaling pathway in c-Rel-mediated Ucp4 expression is not clear at present.

Aerobic glycolysis occurs during brain activation and is characterized by preferential up-regulation of glucose utilization compared with oxygen consumption even though oxygen level and delivery are adequate. Increased lactate production, lactate dispersal

from cells and lactate release from the brain are the major factors underlying aerobic glycolysis (reviewed in [140]). Astrocytes derive energy from both glycolysis and oxidative phosphorylation. Glycolysis and glycogenolysis are essential for astrocytic responses to increasing energy demand by neurons because astrocytic filopodial and lameripodial extensions, which account for 80% of their surface area, are too narrow to accommodate mitochondria [141]. Thus, the change in cell morphology and Ucp4 upregulation through the PKA-Rac1 signaling axis is important for the enhancement of aerobic glycolysis in astrocytes.

6. Sequential activation of CK2 and PKA controls circadian rhythm

Regulation of circadian clock components by phosphorylation plays an essential role in clock functions and is conserved from fungi to mammals. Casein kinases and PKA mediate sequential phosphorylation events in the circadian negative feedback loop [142]. CK2 is an important regulator of Per protein in Drosophila [143]. CK2 binds and phosphorylates Per2, which supports nuclear accumulation of Per2 and affects stability of Per2 in mouse fibroblasts, and inhibition of CK2 activity disrupts circadian rhythm [144,145] (Table 1). Notably, CK2 suppresses activation of $A_{2A}R$ and D1R associated $G_{\alpha s}$ [146], Rac1 [147,148] and NOX [149] thereby downregulates PKA signaling (Table 1).

PKA activation is also important for circadian phase control in mammals [150-152]. PKA activates cAMP-response elements binding proteins (CREBs) by phosphorylation. CREBs are nuclear factors acting as activators and repressors for target gene expression [153]. Notably, the cAMP-PKA-CREB signaling positively regulates gene expression of both BDNF and TrkB receptor in neurons [154,155]. In mouse liver, TrkB mRNA levels peak about 4 h earlier than $p75^{NTR}$ mRNA levels [12]. These results suggest that both BDNF and TrkB are gradually upregulated during the dark/active phase and prepared for the phase change.

It has been shown that there is an autonomous circadian redox oscillation as observed in the *S*-sulfinylation of peroxiredoxins in cells [156] and that there is a cross-talk between circadian redox oscillations and metabolism [157]. The BDNF-mediated sequential activation of CK2-Nrf2 and PKA-c-Rel signaling pathways in astrocytes

provides a cell-type specific example of the coupling of redox rhythm with cellular metabolism. We propose that circadian control of $p75^{NTR}$ expression plays a key role in in rhythmic respiration and redox homeostasis in cerebral astrocytes.

7. Summary: Circadian rhythm switches metabolism via BNDF in astrocytes

Figure 5 summarizes our working model by which circadian control of $p75^{NTR}$ expression drives metabolic switching in the sleep-wake cycle in astrocytes. The BDNF-$p75^{NTR}$-ceramide-CK2 signaling promotes anabolism, favoring storage of energy in the form of glycogen and lipid. This signaling axis activates Nrf2 to back up mitochondrial oxidative phosphorylation to efficiently synthesize ATP when neuronal activity remains at low levels. Ceramide-PKCζ signaling activates RhoA to cause shortening of cell processes thereby reducing the astrocyte's nursing activity. When the $p75^{NTR}$ expression decreases, the BDNF-TrkB.T1-mediated signaling axis in turn activates cAMP-PKA to enhance catabolism. This signaling axis can be enhanced by stimulation of adenosine-A_{2A}R and dopamine-D1R signaling pathways. PKA-mediated signaling specifically activates NF-κB component c-Rel and elongation of processes (Fig. 2 C and 4 B). PKA-mediated signaling enhances production of glucose from glycogen and upregulated Ucp4 increases production of lactate while reducing oxygen consumption in astrocytes to support high neuronal activity.

Recent studies have revealed time-dependent changes in the functional and metabolic interaction between astrocytes and neurons in the SCN of the hypothalamus [158,159], where the rhythm has a diametrically opposite phase compared to other brain areas in mice. Brancaccino et al. [159] monitored intracellular calcium oscillations in SCN neurons and astrocytes and found that they behaved in an anti-phasic manner. The SCN neurons are active during circadian daytime, while SCN astrocytes are active during circadian nighttime. The astrocytes release metabolites in the extracellular space to inhibit neuronal activation during nighttime. Ablation of Bmal1 in the SCN astrocytes lengthened the circadian period of clock gene expression in the SCN, suggesting the importance of astrocytes in determining circadian rhythms [158]. In contrast, BDNF-TrkB signaling or TrkB tyrosine kinase activity in the SCN neurons, which is required in light-induced circadian clock phase-resetting [160,161], is important to

induce daily rhythm of morphological change in astrocytes. Under the normal TrkB function in the neurons, astrocytic contact areas over dendrites of vasoactive intestinal peptide-producing neurons increase roughly two-fold in early daytime (ZT2) compared to mid nighttime (ZT18) in mice [162]. These studies indicate the importance of a time-dependent shift of interaction between astrocytes and neurons for biological timekeeping.

The maintenance of time-phase and amplitude of $p75^{NTR}$ expression together with the rhythmic expression of TrkB.T1 and BDNF serve as important factors for augmentation of both glycogen storage in astrocytes and release of glucose and lactate from astrocytes to support neuronal activity and survival. Disturbances in circadian rhythms and sleep cycles have been considered as symptoms of aging-related neurodegenerative conditions (reviewed in [163-166]. Circadian rhythm dysfunction is often observed in patients with Alzheimer's, Parkinson's and Huntington disease. Clinical studies and experiments in animal models of neurodegenerative disorders have revealed the progressive nature of circadian dysfunction throughout the course of neurodegeneration. As disruption of the circadian rhythm may accompany reduction of glycogen storage in the brain, manipulation of circadian clock and sleep-wake cycles may promote the normal glycogen-glucose cycle and hence healthy brain aging.

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Legend to figures

Figure 1. **Neurotrophin receptor p75^{NTR} is a clock component involved in circadian control of BDNF-mediated metabolism in astrocytes and neurons. A**, Expression of p75^{NTR} is directly controlled by Clock:Bmal1 complex similar to another clock gene Per2 [12]. **B**, Per2 protein expression in the cerebrum has a peak in early light/rest phase and a trough in early dark/active phase. **C**, Astrocytes store glycogen and feed lactate and other metabolites to neurons to support their activities. BDNF coordinates the metabolic interaction between astrocytes and neurons.

Figure 2. **TrkB.T1 facilitates regulation of Rho GTPases**. **A**, TrkB.T1 binds RhoGDI that keeps RhoGTPase in an inactive form [37,40]. BDNF-mediated activation of Rho GTPases requires transfer to membrane, GTP binding and interaction with their effectors. RhoA, Rac1 and Cdc42 are the major Rho GTPases that rearrange actin cytoskeleton. **B**, RhoGDI-associated RhoA and Rac1 can be preferentially transferred to the membrane by phosphorylation of RhoGDI with PKCα and Pak1 kinases, respectively. RhoGTPase-specific Rho-GEFs (guanine nucleotide exchange factors) activate RhoGTPases by exchanging GDP to GTP. **C**, Astrocytes change local and whole cell morphology depending on the activation state of RhoA and Rac1. RhoA facilitates process shortening, while Rac1 and Cdc42 promote process elongation via interaction with their specific effectors. Increase in cyclic AMP elongates cell processes leading to increase in contact areas with neuronal synapses. Activation of p75^{NTR}-ceramide signaling induces RhoA activation.

Figure 3. **Signaling pathways stimulated by BDNF via TrkB.T1-p75^{NTR} receptor complex**. Astrocytes exclusively express a truncated TrkB.T1 receptor that lacks the intracellular receptor tyrosine kinase domain. The low affinity p75^{NTR} inhibits PKA activation [13], and links with neutral sphingomyelinase to produce ceramide upon

stimulation [48]. BDNF stimulation through the receptor complex produces low levels of ceramide, which activates PKCζ. **A**, It is possible that activated PKCζ associates with sequestosome-1 (SQSTM1) and phosphorylates the β-subunits of *Shaker* type potassium $K_v1.5$ channels, causing local membrane depolarization and subsequent Ca^{2+} influx through L-type voltage-activated Ca^{2+} channels, and PKCα and RhoA activation (reviewed in [56]). **B**, Both PKCζ and PKCα phosphorylate and activate casein kinase 2 (CK2), which then phosphorylates PTEN to inhibit its phosphatase activity leading to activation of Akt/PKB signaling [103,104]. Activated Akt phosphorylates and inactivates GSK3β which partly enhances glycogen synthesis. CK2 suppresses $G_{\alpha s}$ activation [146] and thus protein phosphatase-1 (PP1) remains active and enhances glycogen synthesis. CK2 also stabilizes/activates Nrf2 through direct phosphorylation [78,79].

Figure 4. **Functional interaction of TrkB.T1 receptor with adenosine receptor $A_{2A}R$ and dopamine receptor D1R leads to activation of PKA**. **A**, In the absence of $p75^{NTR}$, TrkB.T1 is proposed to interact with adenosine receptor $A_{2A}R$ and dopamine receptor D1R. Stimulation with BDNF, adenosine or dopamine is expected to increase cAMP via receptor-associated $G_{\alpha s}$ protein leading to activation of PKA. PKA plays a key role in glycogen hydrolysis through phosphorylation of DARPP-32, glycogen synthase and glycogen phosphorylase kinase. Phosphorylated DARPP-32 inhibits PP1. PKA also activates NF-κB c-Rel. **B**, Rac1 can be activated by cAMP/PKA signaling [126,127]. Rac1 then activates its effector Pak1 that selectively releases Rac1 from the TrkB.T1-associated RhoGDI (Fig. 2 B). Additionally, PKA phosphorylates RhoA to inactivate it.

Figure 5. Clock regulated $p75^{NTR}$ expression drives daily resetting of energy metabolism in astrocytes. We speculate that cell clock systems maximize $p75^{NTR}$ protein levels between in the late dark/active and early light/rest phase and minimize levels in the early dark/active phase. TrkB.T1-$p75^{NTR}$-mediated signaling enhances RhoA activation, glycogen and lipid synthesis and Nrf2 activation. In contrast, under low $p75^{NTR}$ expression, BDNF-TrkB.T1 signaling activates Rac1 and favors glycogen

hydrolysis, and activates c-Rel. Nrf2 upregulates phase II detoxification and antioxidant enzymes/proteins to support mitochondrial oxidative phosphorylation, while c-Rel upregulates MnSOD and Ucp4 to suppress superoxide production and oxidative phosphorylation in mitochondria leading to enhancement of glycolysis.

Table 1. Neurotrophins-p75^{NTR}-CK2 and CK2-Nrf2 signaling pathways

(1) Neurotrophins activate CK2 and Nrf2

Stimulants	Tissues or Cells	Results	References
NGF	Rat pheochromocytoma PC12	Nrf2 activation	[67]
BDNF, NT-4	Rat hippocampal slices	CK2 activation	[72]
BDNF	Rat hippocampal neurons	CK2 activation	[73]
NGF	Rat hippocampal neurons	p75^{NTR}-dependent CK2 activation	[74]

(2) CK2-dependent Nrf2 activation

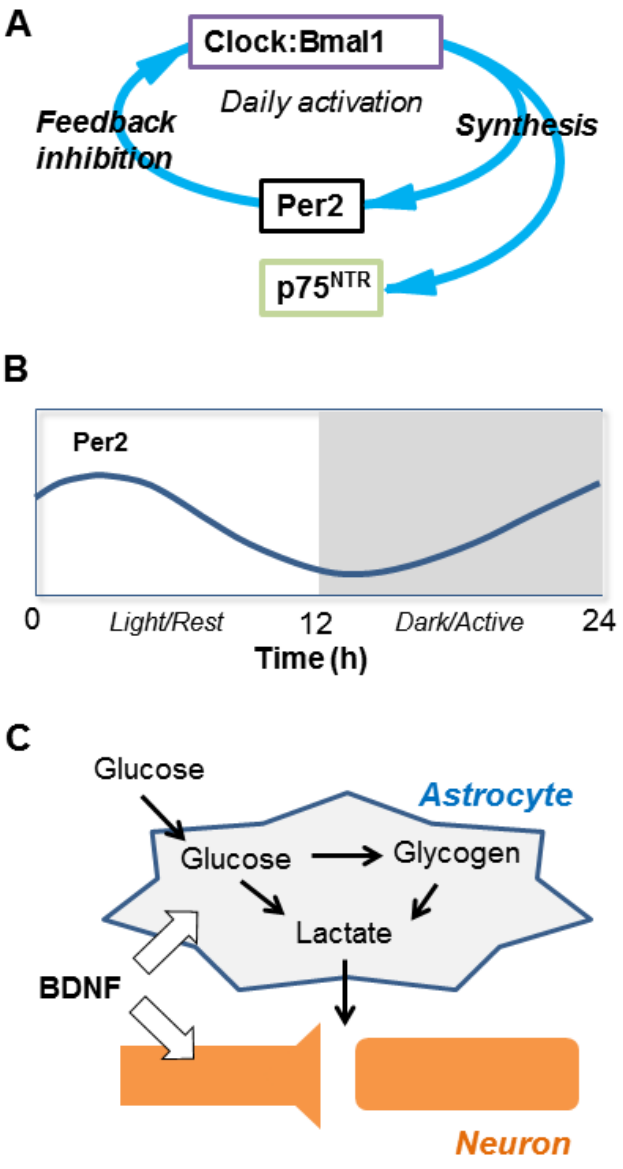
Nrf2 activators or CK2 inhibitor	Cells	Experiments & Results	References
tBHQ, $NaAsO_2$	Human epidermal HaCaT	CK2 inhibitor TBB inhibited Nrf2 phosphorylation/activation	[78]
tBHQ	Human neuroblastoma	CK2 inhibitor DMAT inhibited Nrf2 activation, CK2 phosphorylated Nrf2 *in vitro*	[79]
Oxidized phospholipids, tBHQ	Human umbilical vein endothelial cells	LY294002 and CK2-specific inhibitors but not PI3K-specific inhibitor Wortmannin suppressed Nrf2 activation	[80]
LY294002	Human glioblastoma U251	Down-regulation of Nrf2	[85]

Table 2. Ceramide generation by Nrf2 activators

Nrf2 activators	Cells	Results	References
NGF	Rat sympathetic neurons	$p75^{NTR}$-dependent ceramide production, C6-ceramide replaces NGF for neuronal protection	[92]
Oxidized phospholipid and/or oxidized LDL	Human/rabbit arterial smooth muscle cells	Neutral spingomyelinase-2 activation	[94]
	Human macrophage	Ceramide formation	[95]
	Mouse RAW264.7 cells	Ceramide elevation and apoptosis	[96]
	Human microvascular endothelial cells	Neutral spingomyelinase-2 activation	[97]
resveratrol	Human breast cancer cell	Ceramide generation, growth inhibition and apoptosis induction	[98]
	Human prostate cancer cell	Ceramide generation and growth inhibition	[99]
curcumin	Human colon cancer cell	Robust ceramide generation	[100]
	Human malignant glioma	Ceramide generation and cell death	[101]
	Human leukemia HL cells	Bi-phasic ceramide generation	[102]

Highlights

- Neurotrophin receptor $p75^{NTR}$ is a clock component regulating daily metabolic rhythm
- BDNF-TrkBT.1-$p75^{NTR}$ signaling favors glycogen synthesis in astrocytes
- Stimulation of $p75^{NTR}$ generates ceramide which activates PKCζ
- $p75^{NTR}$-ceramide-PKCζ-CK2 signaling activates Nrf2 in astrocytes
- PKCζ-SQSTM1 complex inhibits $K_v1.5$ currents inducing RhoA activation


A
Clock:Bmal1
Daily activation
Feedback inhibition
Synthesis
Per2
p75NTR
B
Per2
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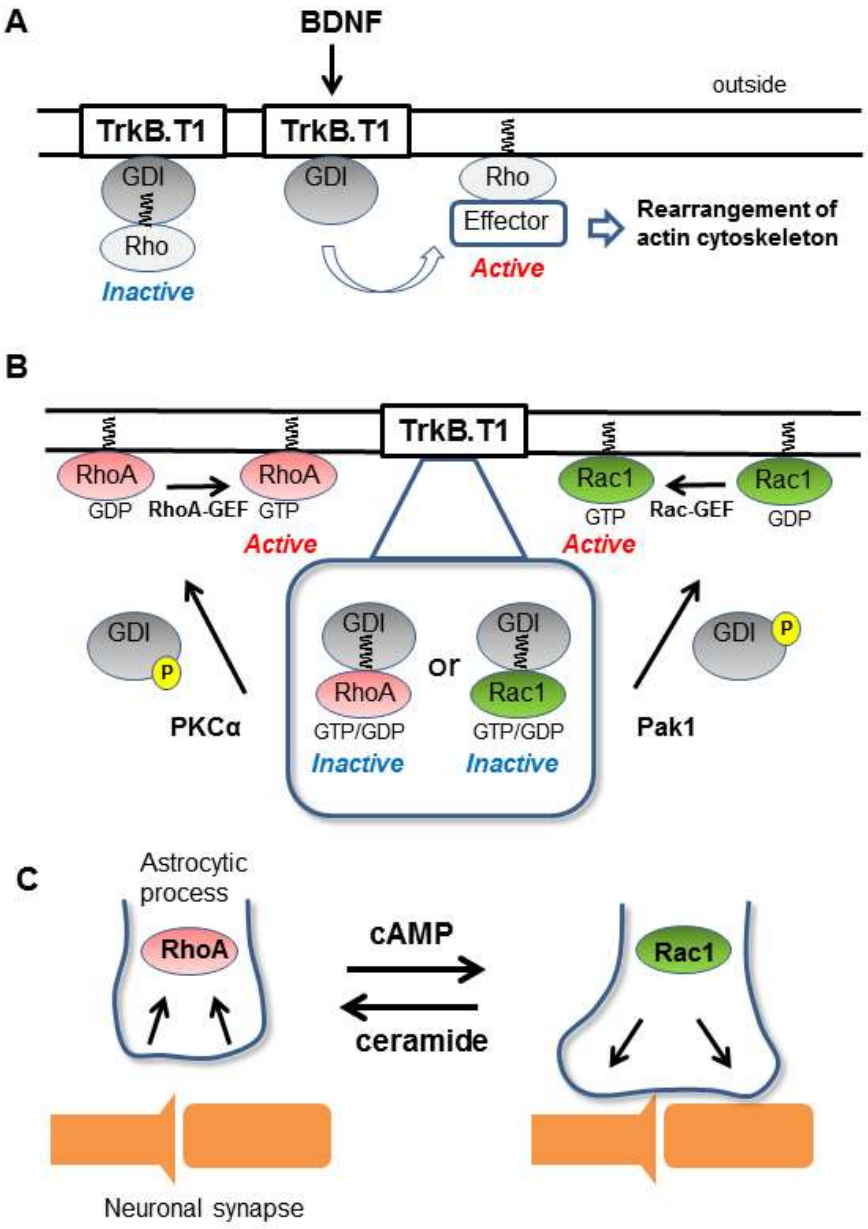
Light/Rest
12
Dark/Active
24
Time (h)
C
Glucose
Astrocyte
Glucose
Glycogen
Lactate
BDNF
Neuron

Fig. 1


A
BDNF
outside
TrkB.T1
TrkB.T1
GDI
Rho
Inactive
GDI
Rho
Effector
Active
Rearrangement of
actin cytoskeleton
B
TrkB.T1
RhoA
GDP
RhoA-GEF
RhoA
GTP
Active
Rac1
GTP
Rac-GEF
Rac1
GDP
Active
GDI
P
PKCα
GDI
RhoA
GTP/GDP
Inactive
or
GDI
Rac1
GTP/GDP
Inactive
GDI
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Pak1
C
Astrocytic
process
RhoA
cAMP
ceramide
Rac1
Neuronal synapse

Fig. 2

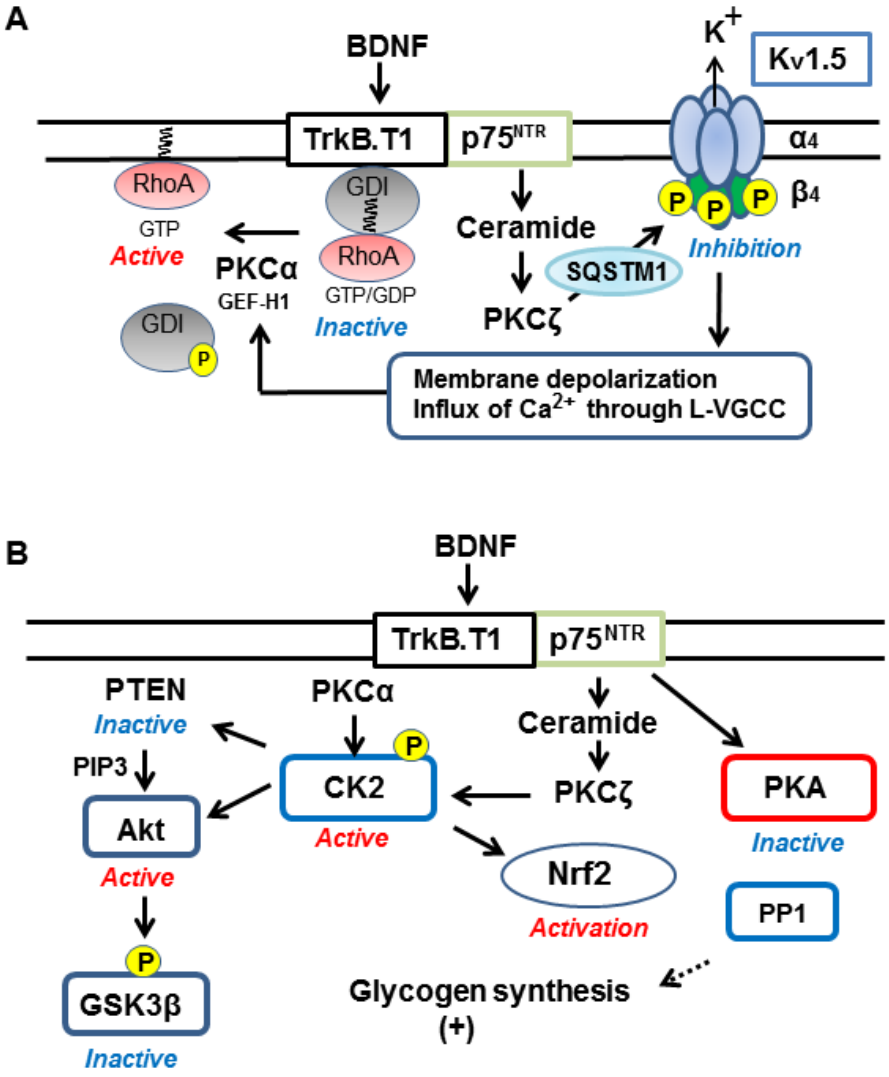
A
BDNF
K+
Kv1.5
TrkB.T1
p75NTR
α4
RhoA
GTP
Active
GDI
RhoA
GTP/GDP
Inactive
PKCα
GEF-H1
GDI
P
Ceramide
SQSTM1
PKCζ
P
P
P
β4
Inhibition
Membrane depolarization
Influx of Ca2+ through L-VGCC
B
BDNF
TrkB.T1
p75NTR
PTEN
Inactive
PKCα
P
Ceramide
PIP3
CK2
PKCζ
PKA
Akt
Active
Active
Inactive
Nrf2
Activation
PP1
P
GSK3β
Inactive
Glycogen synthesis
(+)

Fig. 3

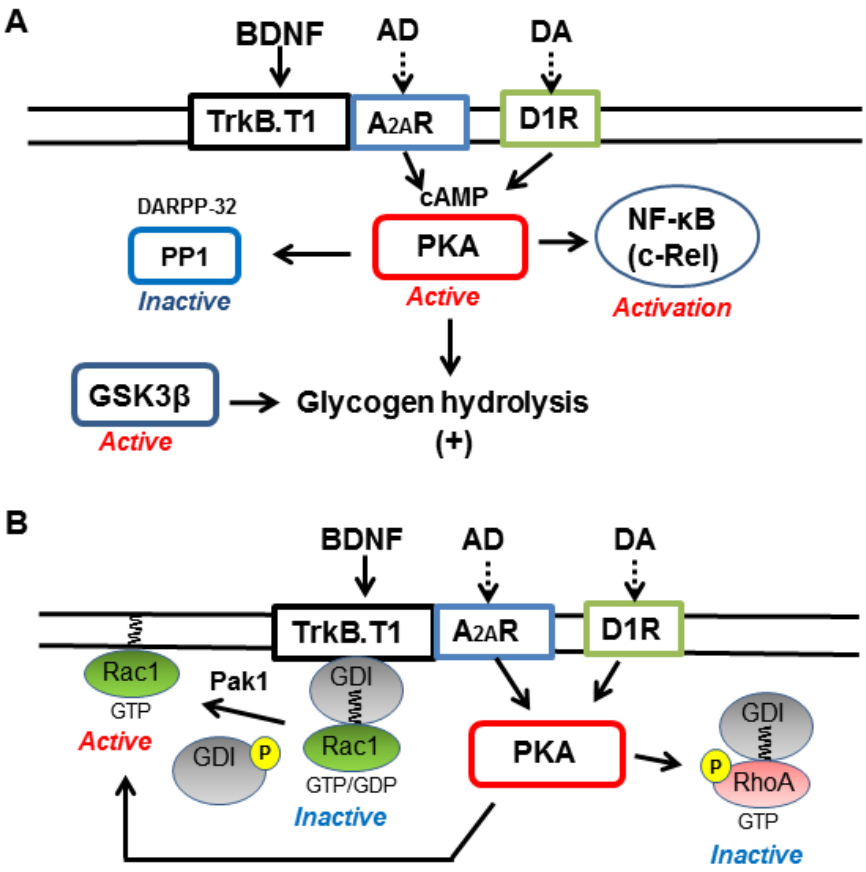
A
BDNF
AD
DA
TrkB.T1
A2AR
D1R
cAMP
DARPP-32
PP1
Inactive
PKA
Active
NF-κB
(c-Rel)
Activation
GSK3β
Active
Glycogen hydrolysis
(+)
B
BDNF
AD
DA
TrkB.T1
A2AR
D1R
Rac1
GTP
Active
Pak1
GDI
GDI
P
Rac1
GTP/GDP
Inactive
PKA
GDI
P
RhoA
GTP
Inactive

Fig. 4

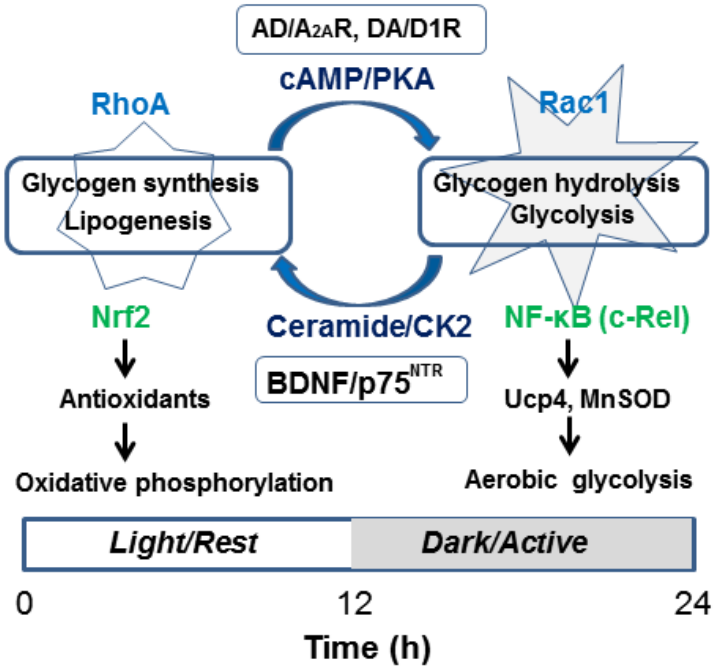
AD/A2AR, DA/D1R
cAMP/PKA
RhoA
Rac1
Glycogen synthesis
Lipogenesis
Glycogen hydrolysis
Glycolysis
Nrf2
Ceramide/CK2
NF-κB (c-Rel)
BDNF/p75NTR
Antioxidants
Ucp4, MnSOD
Oxidative phosphorylation
Aerobic glycolysis
Light/Rest
Dark/Active
0
12
24
Time (h)

Fig. 5